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NEOTENY[1] AND THE SEXUAL PROBLEM

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It has long been known that the larvæ of certain Urodeles sometimes fail to undergo metamorphosis, yet become sexually mature in the larval stage. Perhaps the best known of such cases of neoteny, as this phenomenon is called, is that of the Mexican axolotl, long regarded as a separate species, now known to be an overgrown, sexually mature larva of *Amblystoma tigrinum*. Several other similar cases have been described, however. All neotenous amphibians hitherto reported, with the exception of Allen's (1) thyroidless tadpoles, have been confined to the tailed amphibians, and so far as the writer is aware, the normal occurrence of precocious ripening of the sex cells in larval Anura has never been described. Oddly enough, it is the normal thing, and its occurrence throws considerable light upon the obscure problem of sex differentiation and development in the Anura, which has long puzzled investigators of this subject. It will be recalled that Pflüger (5) reported years ago, that there occurs normally in newly metamorphosed frogs three kinds of individuals, males, females and hermaphrodites, the two latter forms much more numerous in early stages than the males. In the course of further development the hermaphrodites become either definitely male or female, as the sex ratio for adult frogs is approximately 50–50. The investigations of R. Hertwig, Kuschakewitsch and Witschi (2) not only confirmed Pflüger's work, but extended it by showing that anurans apparently first develop solely as females and sexual intermediates, the males only later differentiating from the females and hermaphroditic forms. Moreover, these investigators described in great detail modification of the sex ratios by environmental changes such as extremes of temperature and late fertilization. All of these alleged facts have given rise to the belief that anurans in their sexual development differ greatly from other vertebrates.

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[1] The choice of the word neoteny is perhaps not a fortunate one but because it has come to be associated with the attainment of sexual maturity in the larval stage, it will be employed here in that sense. Literally, neoteny means the prolongation or extension of the period of youth, and it has no necessary relation to sexual conditions.

For several years the writer has been engaged in studying the germ cells of anurans, more especially *Rana catesbiana,* with the object of testing the theories of sex differentiation and development of the Pflüger-Hertwig-Kuschakewitsch school of German investigators. Although at first inclined to admit their contentions, a more careful survey of my material revealed several facts irreconcilable with their views, but which could not be satisfactorily interpreted. Fortunately an opportunity presented itself of working with Professor E. G. Conklin who suggested, after examining my material, that I was probably dealing with a case of precocious ripening of the germ cells in anuran larvæ, *i.e.,* condition stimulating neoteny, using this word in the sense applied above. The suggestion proved correct, and it is a pleasure to acknowledge my indebtedness to Professor Conklin for giving the clue to correct interpretation of the problem and for many other helpful suggestions as well. The present paper is a brief summary of a more extensive and detailed investigation scheduled for later publication.

In *Rana catesbiana* the larval period is very long, some few individuals requiring four seasons to complete metamorphosis, though the usual period is about two years. The sex of larvæ 55–65 mm. in length is not difficult to determine by examination of the gross structure of the gonads, but if such superficial examination is supplemented by a hasty survey of the microscopic appearance of the germ cells, then oddly enough hopeless confusion of the sexes results, and what were apparently males from macroscopic evidence turn out to be apparent females. The sex ratios will vary greatly according to the stress laid by the observer on the gross appearance of the gonads (and it must be admitted that in early stages the gonads of the two sexes are remarkably alike) or upon the cytological evidence as it has heretofore been interpreted. In larvæ of 80–100 mm. length the sex ratio is approximately 50–50 when based on the evidence presented by the gross appearance of the gonads; on the other hand, the cytological criterion, as it has been interpreted, practically does away with males, while most of the animals are apparently female. It is probably due to this erroneous interpretation of cytological conditions that such confusion reigns in the literature regarding sex in anurans.

The germ cells of larvæ 45 mm. and over, both *male* and female, are found in early maturation stages. Such animals are about 8 months of age. Practically all of the female cells are in the leptotene or pachytene stage. In the females the leptotene and pachytene stages do not persist for any length of time, but give place to the period of growth, in which the cells with pachytene nuclei increase greatly in volume, are invested by a follicle of peritoneal cells, and become typical oocytes. Those cells bordering the lumen of the gonad, are first to enter the growth period, and by reason of their great increase in size, fill up the cavity. Around the periphery of the gland a ring of cells with leptotene and pachytene nuclei persists, giving rise later to oocytes of a younger generation. Scattered through the gland are a few oogonia with polymorphic nuclei.

The female gland increases greatly in size due to the growth of the oocytes, becomes much infolded and convoluted by inequalities of growth, thus taking on the characteristics of the typical ovary of the adult. These typical ovaries are to be found in larvæ over 80 mm. long. The gonads probably persist in this condition for several years after metamorphosis, the oocytes growing very slowly. According to the observations of Hertwig and others, the females of *Rana temporaria* and *Rana escu-*

lenta do not become fully mature and ready for copulation until the fifth season after metamorphosis. The writer has captured two females of *Rana catesbiana* two seasons after metamorphosis which were yet sexually immature, hence it seems that the females of this species also require a long period of time in which to develop sexual maturity. The developmental history of the male gonads and germ cells is quite different, and when rightly interpreted fails to show female animals transforming into males and vice versa, or abnormal sex ratios.

It was stated that germ cells of the male larvæ begin their maturation cycle simultaneously with those of the females. This is a very unusual condition and probably unique among the vertebrates though common enough in the invertebrates. It will be recalled that in the vertebrates—for example the mammalia, a very long period of time, sometimes years, separates the maturation cycle of the sexes. In many instances in the female, the initial maturation changes preceding the growth period of the oocyte, occur before birth, whereas the same nuclear changes in the male cells do not make their appearance until shortly before the attainment of sexual maturity. It has become the custom for investigators of sexual conditions in the Anura to use this fact of the early occurrence of maturation stages preceding the growth period of the oocyte in the female as a cytological criterion for differentiating the sexes in the larval stages. Unfortunately this principle, though true enough for other vertebrates is not applicable to Anurans and the result has been hopeless confusion of the sexes because in this group maturation occurs in larval males.

From the period of formation of pachytene nuclei, the history of the sexes is quite different in *Rana catesbiana* and unmistakable if a complete series of larval stages is obtained. In justice to other investigators whose results the writer criticizes as based upon misinterpretation of sexual conditions, it is fair to point out that of all existing species of Anura, *Rana catesbiana* is apparently the only one in which precocious ripening of the male germ cells goes as far as the formation of the maturation division in first year larvæ, and ripe spermatozoa in second year animals. This is of course due to the extraordinarily long larval period. In *Rana temporaria* and *Rana esculenta* the larval maturation changes apparently go only up to and including the pachytene stages before degeneration sets in. In Bufo, the precocious ripening of the sex cells is confined entirely to the cells of Bidder's organ and continues up to the pachytene stage before growth begins.

The male larvæ of *Rana catesbiana* undergo two distinct seasonal maturation cycles as larvæ. The first occurs in young animals 45–60 mm. total length, despite the fact that the germ gland is in an extremely undifferentiated condition. The germ cells develop normally through the leptotene, pachytene, diplotene and tetrad formation stages, but invariably degenerate and go to pieces during the late metaphase or early anaphase of the first maturation division. The centrosomes fragment and the spindle apparatus is aberrant. There are no second maturation divisions, though occasional giant spermatid-like structures may form by the growth of axial filaments from the centrosomes of first spermatocytes. The cells of the first larval maturation cycle degenerate. Through active mitotic division the few primary spermatogonia scattered throughout the gland give origin to those cells which later undergo the second larval sexual cycle. This second cycle occurs near the end of larval life, *i.e.*, usually about two years after hatching. Oddly enough the second maturation cycle is normal, and gives rise to functional spermatozoa in the larvæ, though the efferent ducts of the testes are not yet fully formed. The germ cells and tetrads of the first sexual cycle are aberrant in size and character, those of the second cycle are normal in every way.

The diploid chromosome number of the larvæ is twenty-eight, the haploid number is fourteen. There is no evidence of an accessory chromosome.

Probably the larval sexual cycle just mentioned is an interesting example of a "carrying over" in ontogeny of an earlier phylogenetic condition when the Salientia were sexually mature and normally reproduced as larvæ. It is interesting to note in this connection that male anuran larvæ whose period of metamorphosis is indefinitely postponed, as for example by thyroid extirpation, readily mature sexually, in so far as the production of ripe spermatozoa is concerned.

The male germ cells, unlike those of the female, do not undergo growth, except in relatively rare instances to be described later, and consequently do not fill up the lumen of the gonad. This lumen later is obliterated by the migration into the gonads of cells from the mesentery and possibly from the cortical substance of the adrenal gland. From this ingrowth the testicular interstitium and rete apparatus develops. The efferent tubules at the time of metamorphosis form a connection with the mesonephros. The true sex cords of the testis arise as proliferations of the germinal epithelium, and not as so often claimed for amphibia, as ingrowths from the mesonephros.

This phenomenon of precocious ripening of the male germ cells of *Rana catesbiana* larvæ undoubtedly occurs in other Anura, though is not carried so far as in the bullfrog. The figures of Kuschakewitsch and Witschi show clearly that this condition exists in *Rana esculenta* and *Rana temporaria*. Indeed, it seems more than likely that these writers have mistaken male frog larvæ whose germ cells were in early pseudo-reduction stages, for hermaphrodites and females. The so-called sexually indifferent or sexually intermediate forms of the Pflüger-Hertwig school are very probably male animals whose germ cells show precocious ripening as far as the pachytene stage. This is plainly evident from their photographs, drawings and descriptions. This probable misinterpretation of the cytological data accounts for the transformation of such so-called hermaphrodites into male animals, so minutely described by these investigators. Using the chief criterion of sex differentiation employed by Witschi, *i. e.*, that all germ cells in the larval gonads showing pseudoreduction (leptotene and pachytene stages) are to be regarded as female, the writer obtains in his set of 2,000 animals, 96 per cent. females and only 4 per cent. males. In larvæ of 85 mm. length the percentage of males is zero if this criterion of sexual differentiation is employed. The writer has found so far no evidence that the sex ratios of Anure are any different from those of other vertebrates, and is inclined to regard the confusion concerning sex differentiation and development in anurans as a result of interpretating male animals showing precocious maturation changes of the germ cells as females and hermaphrodites. Sex in the frog does not appear to be nearly so labile and easily influenced as some investigators claim. Professor Hertwig's "late fertilization" experiments are more satisfactorily interpreted on the chromosomal hypothesis of sex determination, than on any other.

One reason so many workers dealing with anurans have regarded these animals as possessing hermaphroditic tendencies is the occurrence of "oocytes," so-called, in the testes of larval and adult frogs and the presence of the peculiar ovary-like structure, the organ of Bidder, in the Bufonidæ. The origin of these apparent oocytes in *Rana catesbiana* has not yet been worked out as completely as the writer could wish; however, enough data has been collected to warrant a tentative explanation of their occurrence in this form and the same data is suggestive as regards the nature of Bidder's organ in the male toad, at least suggestive enough to warrant a reinvestigation of this structure, now generally regarded as a rudimentary ovary.

In male *Rana catesbiana* larvæ, these large oocyte-like cells are of frequent occurrence, and assume this character while in the pachytene stage. Previous to the growth period they are indistinguishable from the other pachytene male cells of the gonad. During the growth stage, which is later followed by their degeneration and disappearance, they are similar in every way to the cells of Bidder's organ. Their follicles are derived from surrounding peritoneal or stroma cells. These follicles are commonly observed surrounding isolated spermatogonia. In certain male gonads a few cells grow to the size of oocytes, and possess yolk nuclei. The presence of yolk, however, is no sex criterion for the male germ cells of many animals form yolk as for instance *Ascaris,* and the apyrene spermatozoa of certain Prosobranchs, and the degenerating cells of the frog.

The larval spermatocytes of the first maturation cycle are in many instances of enormous proportions, scarcely smaller than many organs of Bidder cells near the end of their cycle. It is not impossible that there may be a genetic relation between these two types of testicular elements. This question is reserved for further discussion in a later paper.

The writer is of the opinion that these "oocytes" are of the same nature as the cells of Bidder's organ in *Bufo.* It might be suggested as a possibility worthy of consideration, that in male animals such cells may be of true male character, but owing to the precocious sexual cycle, itself a vestige of a primitive phylogenetic condition when the Anura were sexually mature in the larval form, a few of the germ cells are unable to complete their cycle, and simply grow to an abnormal size, thus assuming the unspecialized character associated with oocytes. These cells degenerate during the second larval maturation cycle when normal sex products are produced. It is rather significant that the whole first larval sexual cycle is abortive in almost every feature. For instance the spermatocytes are abnormally large, the tetrads equally so, the first maturation division never proceeds past the anaphase, the centrosomes fragment, form polyasters, and sometimes axial filaments. Entire cysts of perfectly formed spermatocytes go to pieces in the very act of division, and most of the germinal elements show marked evidences of a deep seated protoplasmic disorganization.

In view of these facts it is possible that the cells of

Bidder's organ in *Bufo,* and the oocytes-like cells of anurans may not be true oocytes, despite their appearance, but may be merely senescent cells, occurring in the course of an abortive and degenerate larval sexual cycle. Bidder's organ on this assumption is the vestigial remains of a primitive sex gland functional when the Bufonidæ reproduced in the larval stage. The functional gonads of present day toads represent recently acquired structures superimposed upon the phylogenetically older and degenerate glands.

In *Rana catesbiana,* a more primitive anuran type than *Bufo,* the entire larval male gonad might with some plausibility be compared to an organ of Bidder in which only a few cells assume the oocyte character whereas the remainder develop a little further, *i.e.,* to the first maturation anaphase, when they too go to pieces. The writer suggests this view of Bidder's organ tentatively, and pending further investigation does not regard himself as irrevocably committed to it. The facts are suggestive, and that is all that can be said at present.

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